

THE HARRIS SLIDE TEST

A MICROFLOCCULATION TEST FOR SYPHILIS WITH CARDIOLIPIN ANTIGEN

BY

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The antigens in common use for the serological diagnosis of syphilis, such as the complement fixation tests and the various flocculation tests, are alcoholic extracts of ox heart usually sensitized with cholesterol. If, however, cholesterol is added to the crude alcoholic extract, false positive reactions are not uncommon. On this account it has become customary to purify the alcoholic extracts of ox heart by first undertaking an extraction with ether, acetone, or ethylene perchloride to remove adventitious substances. But even these purified antigens, which contain the potent phosphatides, are not completely rid of non-specific substances.

Of late years the active lipoid antigen constituents have been subjected to searching studies. It was not, however, until 1941, when Mary C. Pangborn succeeded in isolating a hitherto unrecognized serologically active phospholipid, cardiolipin, from ox heart, that an antigen consisting essentially of chemically pure substances was produced. Used alone as an antigen, cardiolipin is anti-complementary. Mixtures of cardiolipin and purified lecithin have, however, been found highly antigenic, and when cholesterol is added to such mixtures sensitization is attained.

Cardiolipin-lecithin-cholesterol antigens have been used in the complement fixation tests for syphilis by Harris and Portnoy (1944) and by Maltaner and Maltaner (1945), and also for various precipitation methods by Brown (1944, 1945), by Rein and Bossak (1946), and by Kline (1946). The cardiolipin-lecithin-cholesterol ratio has varied in the different techniques employed.

Harris and others (1946) have described a new microflocculation test for syphilis. They use cardiolipin antigen and their test possesses the merit of simplicity. Its findings can be read in a short time. It should, therefore, be suitable for small as well as for large, well-equipped laboratories. It is said to be characterized by satisfactory sensitivity and specificity, and at the same time to possess the merit that all test solutions are standardized.

Harris and others have referred to the test as the V.D.R.L. slide test—an abbreviation of the name of the laboratory in which it was devised—the Venereal Disease Research Laboratory, U.S. Marine Hospital, Staten Island 4, New York. In conformity with current practice in connexion with other flocculation tests, I have ventured to call this the Harris Slide Test in token of its discoverer.

This new test has been carried out at Gade's Institute on 5,556 sera, side by side with the three tests employed as a matter of routine that is, the Bordet-Wassermann, the Kahn standard test, and the Meinicke clarification test No. 2. A short account of the results obtained is given in this communication.

Technique

Briefly, Harris has shown that maximum reactivity, associated with good negative results, was obtained with an antigen in which one part of cardiolipin was combined with nine parts of lecithin and thirty of cholesterol. Thanks to the courtesy of Dr. Harris, we have been supplied with this antigen from the Venereal Disease Research Laboratory. Its formula is as follows:

Cardiolipin	..	0.03 per cent.
Lecithin (approximately)	..	0.27 per cent.
Cholesterol	..	0.9 per cent.
Absolute alcohol	..	q.s.

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The antigen is diluted by the addition of buffered saline solution containing 1 per cent. sodium chloride:

Formaldehyde, neutral, reagent grade ..	0.5 ml.
Secondary sodium phosphate ($\text{Na}_2\text{HPO}_4 + \text{H}_2\text{O}$) ..	0.093 g.
Primary potassium phosphate (KH_2PO_4) ..	0.170 g.
Sodium chloride, A.C.S. ..	10.0 g.
Distilled water ..	1000.0 g.
Final pH 6.0 \pm 0.1	

Antigen and buffered saline are dispensed in screw-cap bottles and stored in the refrigerator.

Preparation of Antigen Emulsion.—Antigen emulsion is prepared by pipetting 0.4 ml. saline to the bottom of a 30 ml. round bottle and adding 0.5 ml. antigen, drop by drop, from a 1.0 ml. pipette (graduated to the tip) while the bottle is being rotated on a flat surface. The last drop of the antigen is blown from the pipette, without touching the saline, and rotation is continued for 10 more seconds. Add 4.1 ml. saline (from 5.0 ml. pipette) place top on bottle, and shake vigorously for 10 seconds. Antigen emulsion is then ready for use and may be used for one day if stored at room or refrigerator temperature.

Preparation of Slides.—Glass slides (2×3 in.) with 12 paraffin wax rings of approximately 15 mm. inside diameters are used. Paraffin wax rings are made by transferring the heated wax to the slides with metal moulds.

Qualitative Serum Test.—The test is performed as follows:

1. Pipette 0.05 ml. heated serum into one ring of a paraffin-ringed glass slide.
2. Add one drop (appr. 1/60 ml.) antigen emulsion into each serum with the help of a 1-ml. syringe fitted with a long needle.
3. Rotate slides for four minutes by hand on a flat surface. This movement should roughly circumscribe a two-inch circle 120 times per minute.
4. Tests are read immediately after rotation.

Reading and Reporting Test Results.—Tests have been read with the help of a powerful lens. Harris recommends microscopic readings at a hundred times magnification. Antigen particles, in properly prepared emulsions, appear as short rods. Aggregation of these particles into large or small clumps is interpreted as degrees of positivity. Clumping of these particles should be reported as follows:

Reading	Report
No clumping or slight roughness ..	Negative (—)
Small clumps ..	Weakly positive (±)
Medium and large clumps ..	Positive (+)

When a magnifying glass is used, there is, as a rule, a definite difference between negative and weakly positive sera. The reading is easy, and only in a few doubtful cases does it depend on the personal judgment of the investigator.

Results

The 5,556 sera are classified in two groups according to the presence or absence of any mention of syphilis in the notes accompanying them. The first group, with evidence of syphilis, included 777 sera with a history and/or clinical signs of syphilis, both treated and untreated. The other group, with no evidence of syphilis, included the remaining 4,779 sera from persons about whom available data provided no evidence of syphilis. In 259 of these there were no data whatever.

Comparisons with Other Tests

Bordet-Wassermann Test (Table I).—Of 777 sera from known cases of syphilis, 636 recorded results which agreed when tested by the Harris and Bordet-Wassermann reactions (81.85 per cent.). Among the remaining 141 sera, there were 37 giving a positive reaction, 63 giving a weakly positive reaction, and 41 a negative reaction to Harris, whereas 76 gave a positive, 23 a doubtful, and 42 a negative reaction to Bordet-Wassermann. The latter, according to these observations, gave a considerably greater number of definitely

TABLE I
COMPARISON OF THE HARRIS SLIDE TEST WITH THE
BORDET-WASSERMANN REACTION

	Evidence of syphilis	No evidence of syphilis
Harris +, B.-W.R. +	281	27
Harris ±, B.-W.R. ±	10	1
Harris —, B.-W.R. —	345	4,690
Total ..	636	4,718
Harris +, B.-W.R. ±	13	1
Harris +, B.-W.R. —	24	3
Harris ±, B.-W.R. +	45	7
Harris ±, B.-W.R. —	18	7
Harris —, B.-W.R. +	31	30
Harris —, B.-W.R. ±	10	13
Total ..	141	61
Grand Totals ..	777	4,779

positive results than did the Harris test. If we also include the weakly positive results among the positives, the two tests show approximately the same sensitivity.

Among the 4,779 sera without syphilis data, 4,718 recorded similar results when tested by the Harris and Bordet-Wassermann reactions (98.72 per cent.). Among the remaining 61 sera there were 4 giving a positive, 14 a weakly positive or doubtful, and 43 a negative reaction to the Harris test, whereas there were 37 giving a positive, 14 a doubtful, and 10 a negative reaction to the Bordet-Wassermann. According to these findings, the Harris test would seem to be somewhat more specific than the Bordet-Wassermann reaction.

Kahn Standard Test (Table II).—Among the sera from known cases of syphilis there were 641 reacting in the same way to the Harris and the Kahn tests (82.5 per cent.). Among the remaining 136 sera there were 57 giving a positive, 45 a weakly positive, and 34 a negative reaction to the Harris test, whereas there were 49 giving a positive, 52 a doubtful, and 35 a negative reaction to the Kahn test. The sensitivity of the two reactions would, therefore, seem to be approximately similar.

Among the 4,779 sera without syphilis data there were 4,731 reacting in the same way to the Harris and the Kahn tests (96.69 per cent.). Among the remaining 48 sera giving conflicting results, there were 9 giving a positive, 11 a

TABLE II

COMPARISON OF THE HARRIS SLIDE TEST WITH THE KAHN STANDARD TEST

	Evidence of syphilis	No evidence of syphilis
Harris +, Kahn +	261	22
Harris ±, Kahn ±	28	4
Harris -, Kahn -	352	4,705
Total ..	641	4,731
Harris +, Kahn ±	36	5
Harris +, Kahn -	21	4
Harris ±, Kahn +	31	3
Harris ±, Kahn -	14	8
Harris -, Kahn +	18	11
Harris -, Kahn ±	16	17
Total ..	136	48
Grand Totals ..	777	4,779

TABLE III

COMPARISON OF THE HARRIS SLIDE TEST WITH THE MEINICKE CLARIFICATION REACTION

	Evidence of syphilis	No evidence of syphilis
Harris +, Meinicke +	288	22
Harris ±, Meinicke ±	17	5
Harris -, Meinicke -	320	4,686
Total ..	625	4,713
Harris +, Meinicke ±	16	4
Harris +, Meinicke -	14	5
Harris ±, Meinicke +	47	4
Harris ±, Meinicke -	9	6
Harris -, Meinicke +	50	35
Harris -, Meinicke ±	16	12
Total ...	152	66
Grand totals ..	777	4,779

weakly positive, and 28 a negative reaction to Harris, whereas 14 gave a positive, 22 a doubtful, and 12 a negative reaction to the Kahn test. These observations suggest that here, too, the Harris is somewhat more specific than the Kahn test.

Meinicke Clarification Test (Table III).—Among the sera from known cases of syphilis there were 625 reacting in the same way to the Harris and the Meinicke reactions (80.44 per cent.). Among the remaining 136 sera there were 30 giving a positive, 56 a weakly positive, and 66 a negative reaction to the Harris test, whereas there were 97 giving a positive, 32 a weakly positive, and 23 a negative reaction to the Meinicke test. It will thus be seen that the Harris test gave much fewer definitely positive results with syphilitic sera than the Meinicke test, whose sensitivity seems, therefore, greater than that of the Harris test.

Among the sera without any data of syphilis there were 4,713 reacting in the same way to the Harris and the Meinicke reactions (96.11 per cent.). Among the remaining 66 sera there were 9 giving a positive, 10 a doubtful, and 47 a negative reaction to the Harris, whereas 39 gave a positive, 16 a doubtful, and 11 a negative reaction to the Meinicke. It would thus seem that the specificity, in contrast to the sensitivity, of Harris is considerably better than that of the Meinicke.

The Three Other Tests in the Various Stages of Syphilis (Table IV).—In the first five groups in this table we find the numbers of the sera from the different clinical stages of syphilis. This grouping of the material has made the numbers within each group rather small, but on the whole it may be claimed that there is a quite good conformity between the results of the tests. In those cases in which there have been conflicting results, it was nearly always one or more of the other tests which failed and not the Harris. Also in the case of primary syphilis, the Harris would seem to be as good as the three other tests. Among the eight cases of primary syphilis with conflicting results there was one in which the Meinicke failed, four in which the Harris did so, five in which the Bordet-Wassermann did so, and six in which the Kahn failed.

In a group by themselves I have included the various forms of syphilis undergoing specific treatment at the time of the examination and the sera received for examination either at the completion of a course of treatment or for the purpose of control during the four ensuing years. I have grouped as latent syphilis the cases which had received specific treatment more than four years earlier without having subsequently received treatment or having presented clinical signs of this disease.

Among 119 cases of latent syphilis examined, there were 22 reacting in different ways to the four tests. Nine of these sera gave positive reactions to three of these tests, and among them there was only 1 in which the Harris failed.

There were greater discrepancies between the different tests when we dealt with treated syphilis than when we did so with the clinical, untreated forms and with latent syphilis. Among 442 treated cases in the various stages of syphilis, there were 103 which reacted in different ways to the respective tests, but 39 of them were Harris-positive. Among the 26 sera giving positive reactions to three tests there were only four in which the Harris failed.

Thus altogether 777 sera from cases of treated and untreated syphilis were examined. In 627 of these sera (80·7 per cent.) the reactions were identical in the four tests, being positive in 326, negative in 301. Conflicting results were obtained with 150 sera (19·3 per cent.). Of the 46 sera giving positive reactions to three tests, 41 were positive to the Harris test. Altogether the Harris was positive in 65 of these 150 sera giving conflicting results.

Of the 777 sera from patients with syphilis data :

391 were positive for the Harris (50·32 per cent.) ;

390 were positive for the Bordet-Wassermann (50·19 per cent.) ;

390 were positive for the Kahn (50·19 per cent.) ;

434 were positive for the Meinicke (55·85 per cent.).

The Three Other Tests of Sera with no Evidence of Syphilis (Table V).—Among the sera from persons with no syphilis data there were 1,544 sent for the control examination of

TABLE IV
COMPARISON OF THE HARRIS SLIDE TEST WITH THE THREE OTHER REACTIONS IN THE DIFFERENT STAGES OF SYPHILIS

Sera from cases of syphilis	No. of sera	Identical results			Conflicting results			
		No. of sera	+	—	No. of sera	Number of positive reactions		
						3	2	1
Congenital	19	13	13	—	6	—	4 (4)	2
Primary	41	33	21	12	8	2 (2)	4 (1)	2 (1)
Secondary	47	46	46	—	1	1 (1)	—	—
Tertiary	76	69	69	—	7	5 (5)	2	—
Cerebral	33	30	29	1	3	3 (3)	—	—
Latent	119	97	29	68	22	9 (8)	6 (1)	7
Treated	442	339	119	220	103	26 (22)	43 (7)	34 (10)
Total ..	777	627	326	301	150	46 (41)	59 (13)	45 (11)

()—Number of positive Harris Slide Tests

pregnant women in accordance with Norwegian law. Among these sera were 10 giving positive reactions to all the tests, and 15 giving conflicting results, with the reaction to the Harris positive in only 2 cases.

There were 530 sera from healthy persons needed as blood donors or requiring health certificates or the like. In 526 of these sera the reactions were negative to all the tests. Among the remaining four sera giving conflicting results, there was one positive to the Harris.

It is said that there is a tendency for false positive reactions to be associated with diseases of the lungs and acute infections, and the sera from such cases have, therefore, been grouped separately. Of the 11 sera from patients with such diseases and showing conflicting results, 4 were positive to the Harris.

Among 2,033 sera from patients suffering from various diseases without syphilis data there were 53 giving conflicting results with the four tests. Only 7 of these sera were positive to the Harris.

No data whatever were available in connexion with 259 sera, among which one might, therefore, expect to find cases of known syphilis, treated or untreated. Twelve of these sera gave positive reactions to all 4 tests, and 3 were positive to 3 tests, which in two cases was the Harris test. Two were positive to the Bordet-Wassermann and to the Meinicke, but negative to the Harris and the Kahn. Six were positive to only one test which in 3 cases was the Bordet-Wassermann, in 1 the Meinicke, and in 2 the Harris.

Altogether there were 4,779 sera without data indicative of syphilis. Among them were 4,685 (98.03 per cent.) giving identical reactions to the four tests, 28 positive and 4,657 negative. Among the remaining 94 sera (1.97 per cent.) with conflicting results there were 18 positive to the Harris. Half of these sera with conflicting results were positive to only one test, which in 6 cases was the Harris.

Among the 4,779 sera without syphilis data there were :

- 46 positive (or doubtful) to the Harris (0.96 per cent.) ;
- 79 positive (or doubtful) to the Bordet-Wassermann (1.65 per cent.) ;
- 62 positive (or doubtful) to the Kahn (1.30 per cent.) ;
- 82 positive (or doubtful) to the Meinicke (1.72 per cent.).

Comparison of the Strength of the Harris Slide Test with that of the Other Tests (Table VI).—The syphilitic sera giving a definite positive or a weak positive reaction to all the tests, or a negative reaction to all, were therefore classed as identical results. Among these sera there were more which gave markedly positive reactions to the Bordet-Wassermann and to the Meinicke than to the Harris and the Kahn. When there was a conflict of the reactions in sera from patients with a history of syphilis, Meinicke's was the test which yielded the greatest number of definitely positive results, whereas here also the Harris occupied third place among the four tests.

TABLE V

COMPARISON OF THE HARRIS SLIDE TEST WITH THE THREE OTHER REACTIONS IN SERA WITH NO EVIDENCE OF SYPHILIS

Sera from	No. of sera	Identical results			Conflicting results			
		No. of sera	+	—	No. of sera	Number of positive reactions		
						3	2	1
Pregnancy	1,544	1,529	10	1,519	15	3	1	11 (2)
No diseases	530	526	—	526	4	—	1 (1)	3
Diseases of the respiratory tract ..	228	221	4	217	7	3 (2)	3 (1)	1
Acute infections	185	181	1	180	4	1	1 (1)	2
Various other diseases	2,033	1,980	1	1,979	53	6 (2)	23 (3)	24 (2)
No notes	259	248	12	236	11	3 (2)	2	6 (2)
Totals ..	4,779	4,685	28	4,657	94	16 (6)	31 (6)	47 (6)

()—Number of positive Harris Slide Tests

TABLE VI
STRENGTH OF REACTION IN THE VARIOUS TESTS

Methods	Identical results			Conflicting results			
	+	±	—	+	±	—	
Harris	281	45	301	37	28	85	Evidence of syphilis
B.-W.R.	308	18	301	49	15	86	
Kahn	276	50	301	34	30	86	
Meinicke	300	26	301	85	23	42	
Harris	22	6	4,657	9	9	76	No evidence of syphilis
B.-W.R.	27	1	4,657	37	14	43	
Kahn	21	7	4,657	15	19	60	
Meinicke	21	7	4,657	40	14	40	

Altogether among the syphilitic sera there were 318 giving a definitely positive reaction and 73 a weakly positive reaction to the Harris, whereas the corresponding figures for the Meinicke were 385 and 49, for the Bordet-Wassermann 357 and 33, and for the Kahn 310 and 80 respectively.

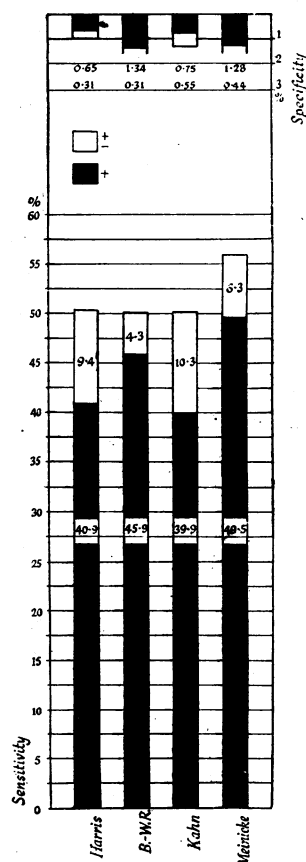
Among sera without syphilis data there were 31 giving a definitely positive reaction and 15 a weakly positive reaction to the Harris, whereas the corresponding figures for the Kahn were 36 and 26, for the Meinicke 61 and 21, and for the Bordet-Wassermann 64 and 15 respectively. As pertinent data were either defective or, in the case of 259 sera, altogether lacking, there may have been some syphilitic sera among them. Even though this is a possibility, the specificity of the Harris test must be considered to be very satisfactory.

The sensitivity and specificity of each reaction is shown in the graph.

Discussion

The comparative investigations of the Harris slide test and the three other syphilis tests carried out as a matter of routine at Gade's Institute on current material has shown that the former possesses a sensitivity on a level with that of the Kahn standard test and the Bordet-Wassermann test, whereas the Meinicke clarification test has yielded a greater number of positive results. If, however, a distinction is made between definite and weak positive results, the Bordet-Wassermann test also gives a greater number of definite positive results than the Harris slide test, while the Kahn standard test is at about the same level with it.

In a preliminary report on the test, Harris



GRAPH.—Sensitivity and specificity of Harris, Bordet-Wassermann, Kahn, and Meinicke reactions.

and others (1946) recommended microscopic readings at a hundred times magnification. We adopted this microscopic system in our preliminary investigations, which ante-dated the investigations reported in this communication; but we found that it was difficult in several sera to decide whether the reaction was negative or weakly positive, as there was in these sera a "roughness" difficult to distinguish from small clumps. Accordingly, we read off the results with a lens immediately after rotation. We found this procedure satisfactory; it is easier and quicker than a microscopic reading. It is, however, possible that we recorded as weakly positive sera which on a microscopic reading would have been read off as definitely positive. As, however, we have found that what is most important is to distinguish between negative and weakly positive results, we have continued to use a lens and have thereby achieved results with satisfactory sensitivity and very good specificity.

The reading itself, as practised in our laboratory, is therefore easy and only to a slight degree dependent on a personal opinion in borderline cases. Most of the definitely positive results are also directly visible to the naked eye.

Except in Oslo, Norway is equipped with few laboratories which undertake diagnostic serological reactions in syphilis, and the lines of communication are still in many places so defective that samples of blood take several days to reach this laboratory. Hence a discussion of the possibility that the various hospitals lacking laboratories for the serological diagnosis of syphilis could profitably undertake a simple serological test for syphilis with good sensitivity and specificity as a routine measure. They would thus have to send to a special serological laboratory only those sera which gave a positive or questionable reaction to such a test. This would, however, be a retrograde step in the serological study of syphilis.

At most laboratories it is now the custom to carry out several diagnostic serological syphilis reactions side by side. These reactions supplement one another, and thus make a diagnosis more certain than when it depends on only one reaction. Hitherto no reaction has been devised which is so pre-eminent that it renders all other reactions superfluous. There are plenty of examples showing that even the

most delicate reaction can fail to indicate definite cases of syphilis when other reactions, in other respects found to be less delicate, have been positive.

The need for blood donors has of late years constantly grown. But there are many examples of infection with syphilis by blood transfusion. The serological examination of blood donors for syphilis before a blood transfusion has, therefore, been adopted as a routine measure. This examination should include several syphilis reactions, but when the same donor is used at intervals of only a few months, and it is not possible on each occasion to undertake a complete serological examination for syphilis at a laboratory, it would be better to carry out a single reaction than to omit it. Under such conditions the Harris slide test, with its simple and rapid technique, should prove useful provided it is undertaken by a serological expert.

Conclusion

The Harris slide test is useful in the diagnosis of syphilis.

After comparative investigations with the Bordet-Wassermann reaction, the Kahn standard test, and the Meinicke clarification test in 5,556 sera, we have found the sensitivity of the Harris test satisfactory and its specificity good.

The antigen in the Harris slide test consists of chemically pure lipoids. All the test solutions are standardized. The test is rapid and easily carried out. It is easy to read off the results, and only in a few doubtful cases must personal judgment be depended on.

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